# V. HEALTH EFFECTS IN ANIMALS

## Introduction

Animal studies on chloramines are limited. Few health-related studies have been conducted on chloramines in drinking water. Since chloramines may be used as an alternate means of disinfection, more studies are being conducted. Summaries of the acute, subchronic and chronic oral studies are presented in Tables V-1 and V-2.

## Short-Term Exposure

Because individuals undergoing long-term hemodialysis have been shown to develop hemolytic anemia when water containing monochloramine ($NH_2Cl$) has been inadvertently used in the dialysis treatment, hematologic parameters have been examined in several studies. Moore et al. (1980) exposed 12 male A/J mice per group for 30 days to a bicarbonate buffered solution (pH 8.9) of monochloramine in drinking water at concentrations of 2.5-200 mg/L. They found no statistically significant (two-way analysis and Kruskal-Wallis one-way analysis of variance) changes in nine measured blood parameters; however, hematocrit did rise slightly with increasing levels of monochloramine. The authors concluded that monochloramine ingested by A/J strain mice did not appear to produce hemolysis even at very high dose levels.

Abdel-Rahman et al. (1984) investigated the toxicity of $NH_2Cl$ in male Sprague-Dawley rats. Acute exposure to a single dose at 10, 20 or 40 mg/L $NH_2Cl$ induced a statistically significant ($p<0.05$) increase in blood glutathione levels within 30

minutes after administration of a 3 mL aqueous solution by gavage. On the other hand, chronic administration at 1, 10 or 100 mg/L doses in drinking water induced a statistically significant ($p<0.05$) decrease in glutathione after 4 months of exposure at the 1 and 100 mg/L dose levels. The difference in glutathione blood levels following subchronic versus chronic exposures may be due to different physiologic responses such as 1) an increase in reduced glutathione levels resulting in increased RBC glutathione reductase activity in subchronically exposed animals, and 2) a decrease in reduced glutathione levels in chronically exposed animals, which is due to depletion of glutathione body stores.

Bercz et al. (1982) studied the toxicity of monochloramine administered in drinking water to 5 adult males and 7 adult female African Green monkeys with body weights ranging from 3.0-5.7 kg. Monochloramine was administered for 6 weeks at 100 mg/L. The authors estimated the mean daily dose to be ~10 mg/kg/day. Such ingestion of monochloramine had no detectable effect in 18 hematologic tests on the 12 monkeys, including red cell GSH levels. No evidence of thyroid suppression was detected in serum. Maziarka et al. (1976) also found that rats exposed for 9 months to 9.0 mg/L chloramines showed no observable hematologic effects. However, the study was not a dose-response type and many of the blood parameters reflecting oxidant stress were not measured.

In a review of alternate disinfectants Bull (1980) reported the results of a 45-day study in which 10, 50 or 100 mg/L (1.4, 7.0 and 14.0 mg/kg assuming rats consume 0.14 L/day) monochloramine in drinking water was administered to laboratory rats. Body

weight gain and hematologic parameters in exposed animals did not differ significantly from control animals. The only significant finding was a decrease in the amount of methemoglobin present in the blood at 100 mg/L monochloramine. This is the opposite of what was expected. There were no signs of overt toxicity (Bull, 1980). Details of this study were not reported.

A subchronic 30-day toxicity test was performed on groups of 10 male and 10 female CD-1 mice using concentrated drinking water samples collected at a pilot scale drinking water treatment plant using monochloramine for disinfection purposes (Miller et al., 1986). The residual monochloramine level was 2.1 mg/L before concentration of organics by reverse osmosis. Samples were concentrated (100x or 400x) before toxicologic testing. Post exposure, the mice were examined for gross pathologic changes. Body weights were not significantly different from controls. There was a statistically significant increase ($p<0.05$) in liver-to-body weight ratios of females and a statistically significant decrease ($p<0.05$) in the lung weights of males and females at 100x. The brain and kidney weights were significantly decreased ($p<0.05$) in males at 100x. At 400x the kidney and liver weights of males were significantly decreased ($p<0.05$) and the ovary weights of females were significantly increased ($p<0.05$). The authors conclude that these data did not reveal any overt toxicity; therefore, histopathologic examinations of major organs was not performed.

A draft report by GSRI (1981) investigated the effects following exposure to monochloramine in Fischer 344 rats and B6C3F1 mice. Rats (10/sex/group) were

administered concentrations of 0, 25, 50, 100, 200 and 400 ppm (25, 50, 100, 200 and 400 mg/L) monochloramine in drinking water ad libitum for 91 days. Using water and food consumption data provided in the report, corresponding doses were calculated to be 2.5, 4.9, 10.2, 18.8 and 40.7 mg/kg/day for males and 3.8, 6.5, 13.8, 26.6 and 53.9 mg/kg/day for females. No animals died during the course of the study. After 25 days the buffer system for monochloramines was changed because of a palatability problem at the high-dose levels. Terminal body weight gains in male rats decreased at the 200 and 400 ppm levels by 13 and 24%, respectively, when compared with their controls. Female rats gained 8 and 14% less weight than their controls at the 200 and 400 ppm levels, respectively. The decrease in the percentage of body weight gain was most marked at 0-4 weeks and appeared to be related to the palatability of the 200 and 400 ppm solutions. Absolute liver weights showed a trend toward reduced liver size; however, when liver weights were expressed as a percentage of total body weight no significant differences were observed. Protein excretion increased in male rats when 200 and 400 ppm monochloramine was administered. Microscopic examination of tissues at the 400 ppm level did not reveal any treatment-related lesions. This study identified a NOAEL for rats of 100 ppm (10.2 mg/kg/day). (Confidence in this study is low because of questions regarding the conduct of this study, the histopathologic evaluations and lack of corroboration of its findings.)

Similarly, mice (10/sex/group) were administered concentrations of 0, 25, 50, 100, 200 and 400 ppm (25, 50, 100, 200 and 400 mg/L) monochloramines in drinking water ad libitum for 91 days. Using water and food consumption data provided in the report

corresponding doses were 4.9, 8.3, 14.5, 31.3 and 50.7 mg/kg/day for males and 7.7, 12.1, 21.9, 34.6 and 88.5 mg/kg/day for females. During the course of the study no animals died. The buffer system for monochloramines was changed after 25 days since palatability was a problem at the high dose levels. Male and female mice gained less weight than the controls at the 200 and 400 ppm levels. There was a reduction in absolute liver weights and liver-to-body weight ratios in male mice at the 400 ppm levels and in female mice at $\geq$100 ppm. Histopathologic observations revealed necrotic changes at the low doses and mild to moderate cytologic alteration in the livers of male mice administered 200 and 400 ppm. Chronic liver inflammatory changes occurred at 100, 200 and 400 ppm in female mice and to a lesser extent in male mice at the 100 ppm level. At concentrations of 100, 200 and 400 ppm increased frequency of mitotic figures, hypertrophy and bizarre chromatin patterns occurred in males and in one female at 200 ppm. This study identified a NOAEL for mice of 50 ppm (8.4 mg/kg/day).

In a more recent study, Daniel et al. (1990) administered 0, 25, 50, 100 and 200 mg/L monochloramine to male and female Sprague-Dawley rats (10/sex/dose) in their drinking water for 90 consecutive days. Using food and water consumption data provided in the report, corresponding doses were calculated to be 0, 1.8, 3.4, 5.8 and 9.0 mg/kg/day for males and 0, 2.6, 4.3, 7.7 and 12.1 mg/kg/day for females. A control group received distilled water buffered with sodium bicarbonate to a pH of 8.0-8.5. Mortality, clinical signs, body weight, organ weights, food consumption, hematology, clinical chemistry, gross pathology, and histopathology were examined. Water consumption was significantly decreased at all monochloramine doses. At 200 mg in males water

consumption was ~31% of controls and in females at 200 mg water consumption was ~34% of controls.

Food consumption was significantly reduced in males at the highest dose tested. In males there was a significant reduction in body weight gain at doses of 50 mg/L and higher. The reduced body weight gain in females was significant only at 200 mg/L. For males and females the average weight gain at 200 mg/L was ~51% of controls. At the 200 mg/L dose, reductions in the absolute weight of liver and spleen were observed in both sexes. In females, the absolute weight of the thymus was also decreased as was the weight of the lung and heart of the males. Also at 200 mg/L, females had increased relative kidney and decreased relative liver weights while males had increased relative brain, testes and kidney weights.

At 100 mg/L males had significantly decreased absolute liver weights and at 50 mg/L males had decreased absolute lung weights. At the 25 mg/L dose, there was no significant effect on absolute organ weights in either sex. The authors concluded that the 100 mg/L dose is considered the NOAEL. Although reductions in organ weights appeared to be dose-related in males, subsequent histopathologic examination did not reveal any target organ or treatment-related changes. Although reduced RBC count (200 mg/L) and decreased calcium levels in males were significant, the authors did not consider them biologically significant, dosage-related or within the normal range for rats of this age and strain.

Daniel et al. (1991) administered monochloramine in drinking water for 90 days to male and female B6C3F1 mice (10/sex/dose) at 0, 12.5, 25, 50, 100 and 200 mg/L. Using food and water consumption data provided in the report, corresponding doses were calculated to be 0, 2.5, 5.0, 8.6, 11.1 and 15.6 mg/kg/day for males and 0, 2.8, 5.3, 9.2, 12.9 and 15.8 mg/kg/day for females. Mortality, clinical signs, body weight, organ weights, food consumption, hematology, clinical chemistry, gross pathology and histopathology were examined. Food consumption was decreased in males and females at the two highest dose levels with a statistically significant decrease in females. Water consumption was decreased in all treated groups with statistically significant decreases in female mice at all treatment levels and male mice at the two highest levels.

In males there was an increase in lymphocytes at 100 and 200 mg/L and in females there was a decrease in neutrophils at 100 mg/L and a decrease in MCV in all dose levels. None of the hematology results were considered to be dose related. Alk-P was decreased in males at all concentrations, statistically significant at 25, 50 and 100 mg/L, while AST was increased in females at all dose levels, and statistically significant at 50 and 200 mg/L.

There were significant reductions in body weight gain in males at the three highest dose levels, and at the two highest dose levels in females. Significant decreases in water consumption occurred in all doses in female mice and at the two highest dose levels in males. Significant reductions in absolute organ weights were evident in males and females drinking 200 mg/L monochloramine. In male and female mice, liver and heart

weights were decreased at the two highest dose levels, and relative and absolute spleen weights were decresed in the two highest doses for females. At the highest dose, the kidney and lung weights were decreased in males and females; also at the highest dose, absolute testes and spleen weights were decreased in males. When analyzed relative to body weight, brain, kidney, lung and testes in males and brain and kidney in females were significantly increased at the highest dose compared with the control value. Based on relatively minor changes at 100 mg/L of monochloramine, including <10% depression of body weight and the greater changes at 200 mg/L (19-25% decreased body weight), the 100 mg/L dose (12.9 mg/kg/day) can be identified as a NOAEL in this study.

No compound-related gross or microscopic lesions were observed, and no target tissues were identified in the monochloramine-treated animals.

Exon et al. (1987) administered monochloramine at 0, 9, 19 and 38 ppm (mg/L) in the drinking water of male Sprague-Dawley rats (12/sex/dose) from weaning to 12 weeks of age (9 weeks of exposure). Based on reference body weight and water consumption values for subchronic exposure (U.S. EPA, 1986), the corresponding intake of monochloramine was 0, 1.3, 2.6 and 5.3 mg/kg/day. Following treatment, the test animals were assessed for immune competence. Parameters of immunity measured were spleen and thymus weights, antibody production, delayed-type hypersensitivity (DTH) reactions, natural killer cell (NKC) cytotoxicity, oxidative metabolism response and phagocytosis by macrophages and production of two immunoregulatory cytokines, interleukin 2 (I12) and prostaglandin E2 (PGE2). Significant ($p<0.05$) reduction of spleen weights (38 ppm),

decreased antibody synthesis (9 and 19 ppm) and augmented PGE2 production (19 and 38 ppm) were reported. The authors state that these results indicate that B lymphocyte, as well as macrophage function may be adversely affected. While monochloramine appears to exert immunotoxic effects, the biologic importance of effects on the immune system as a factor in the toxicity of monochloramines is not clear. In addition, there is a lack of correlation among various endpoints examined, as well as deficiencies in some of the methodologies (i.e., the use of the ELISA test for antibody analysis). Based on these considerations, a NOAEL or LOAEL cannot be determined from this study.

Since pigeons may be susceptible to cardiovascular disease, Revis et al. (1986) investigated the relationship of monochloramine to plasma cholesterol and thyroid levels. Groups of 12 male white carneau pigeons (age 3-4 months) were fed altered diets and drinking water containing either 0 (deionized water), 2 or 15 ppm monochloramine *ad libitum* for 3 months. The treatment diets consisted of either (A) a diet reduced to 0.35% calcium (80% of the minimal daily requirement for a pigeon) i.e., a normal diet, or (B) a diet reduced to 0.35% calcium with the addition of 10% lard and 0.5% cholesterol, i.e., a high cholesterol diet. The controls were given either diet and deionized water *ad libitum*. Treated drinking water was prepared and changed daily. At 1-month intervals blood samples were collected and plasma levels of cholesterol and $T_4$ were determined. The study reported that plasma $T_4$ levels were significantly decreased in pigeons fed a normal diet (A) or high cholesterol diet (B) and drinking water containing 15 ppm monochloramine. Following a 3-month exposure to diet B and receiving either deionized water or water containing 15 ppm monochloramine, plasma cholesterol levels were

1266±172 and 2049±212 mg/dL, respectively, a difference of 783 mg/dL. Significant increases in plasma cholesterol were also observed in pigeons at the 2 ppm level compared with deionized water and when both groups were fed only the calcium-deficient diet (A). No significant changes were observed in the comparative 15 ppm diet (A) group. $T_4$ levels were not significantly altered when 2 ppm monochloramine and diet (A) were administered. There was no clear dose-response effect for plasma cholesterol observed in any of the treatment groups. Thus, factors associated with the effects of monochloramine on plasma cholesterol are not known. The authors suggested that the changes in plasma cholesterol may be mediated by products formed when monochloramine reacts with organic matter in the upper GI tract. Pigeons may or may not mimic humans in thyroid function, so the significance of effects in the pigeons relationship to humans is unknown. Also, pigeons were maintained on a calcium deficient diet in order to depict an average human diet. It is not known if pigeons metabolize calcium in the same manner as humans.

**Other Short-term Effects**

Robinson et al. (1986) treated female Sencar mice (5/dose) with aqueous solutions of monochloramine by whole-body exposure (except head) for a 10-minute period for 4 days to assess hyperplastic effects. The backs of the animals were shaved 3 days before treatment of 1, 10, 100 and 1000 mg/L of monochloramine. Animals were sacrificed the day following the last treatment and skin thickness measured. TPA (12-o-tetradecanoylphorbol13-acetate) was applied at a dose of 1.0 $\mu$g in 0.2 mL acetone/mouse to the positive control mice. Epidermal thickness on the fifth experimental

day measured ~32.8 $\mu$m in the positive controls. The epidermal layer in control animals given water measured ~15.4 $\mu$m. Exposure to 1, 10, 100 and 1000 mg/L monochloramine decreased epidermal thickness (~14.0, 14.4, 13.1 and 13.6, respectively); however, this was not significant when compared with controls. In addition, cell counts were not increased at any of the doses when compared with controls.

Flour bleached with trichloramine administered in the diet has been shown to produce "canine hysteria" or "running fits" in dogs. However, one study suggests that this is a species-specific phenomenon for dogs (Mellanby, 1946; Silver et al., 1947a,b,c; Newell et al., 1947) and does not affect humans (Pollock, 1949). Trichloramine is formed in waters at high chlorine-to-ammonia ratio concentrations and at lower pHs than normally found in drinking water.

**Long-Term Exposure**

Abdel-Rahman et al. (1984) conducted a study to investigate the toxicity of $NH_2Cl$ in drinking water (see Table V-2). Groups of four male Sprague-Dawley rats drank either 0, 1, 10 or 100 mg/L $NH_2Cl$ in deionized water daily for $\leq$12 months. The levels of chlorine, dichloramine and trichloramine in the $NH_2Cl$ solution were <1%, <1% and 0%, respectively, of the total $NH_2Cl$ added. Food was available ad libitum and body weight was measured during the treatment. Cardiac puncture and collection of heparinized blood was performed at 2, 4, 6, 8, 10 and 12 months after treatment. Hematologic parameters, blood glutathione (GSH) levels and osmotic fragility were monitored throughout the treatment period. $^3$H-Thymidine incorporation was studied in liver, kidney,

testes, intestinal mucosa and spleen after 3 months of treatment. Increased incorporation of $^{3}$H-thymidine was observed in the kidney and spleen at 1 and 10 mg/L and in testes at 100 mg/L at 3 months; other time periods were not examined. Body weights were significatnly reduced after 3 months of treatment at 100 mg/L and remained lower than controls throughout the experiment. Blood levels of chloroform monitored during the study were no different than in untreated rats. Results varied over the 12-month study period, but at 6 and 12 months after initiation of the study statistically lower GSH levels were observed at all doses. After 3 months of treatment significant decreases in RBC count and hematocrit were observed at the higher dose levels. A reduction in hemoglobin concentration and mean corpuscular hemoglobin occurred at 100 mg/L after 10 months of treatment. The decrease in blood GSH could be due to a protective role by GSH against damage caused by oxidants. The health significance of these types of changes is uncertain. Furthermore, results were analyzed by inappropriate statistical methods and a number of "significant" changes in hematologic parameters relative to control values were identified that had no consistent relationship with dose and were not observed consistently throughout the period of exposure.

The NTP (1990) conducted two studies to determine the potential chronic toxicity or carcinogenic activity of chloraminated drinking water. In the first study F344/N (70/sex/dose) rats were administered chloramine for 2 years at doses of 0, 50, 100 and 200 ppm in pH 9 buffered charcoal filtered deionized drinking water. These doses were calculated on the basis of a time-weighted average to be 0, 2.1, 4.8 and 8.7 mg/kg/day for male rats and 0, 2.8, 5.3 and 9.5 mg/kg/day for female rats. There was a dose-related

decrease in the amount of water consumed by both sexes; this decrease was noted during the first week and continued throughout the study. Food consumption of treated rats was the same as the controls with males consuming more. Mean body weights of 200 ppm dosed rats were lower than their control groups. However, mean body weights of rats receiving chloraminated drinking water were within 10% of controls until week 97 for females and week 101 for males. At the end of the study all animals were given a complete histopathologic examination. The authors determined that no clinical changes were attributable to chloraminated drinking water. Survival of rats receiving chloraminated drinking water was not significantly different than controls except that, for the 50 ppm dose groups, survival was greater than that of controls.

Groups of rats (10/sex/group) were predestined for incremental sacrifice and evaluation at 14 and 66 weeks. At these times a complete hematologic examination and necropsy were performed on all animals and histopathologic examinations were performed on all control and high-dose rats. In F344/N rats at the 14-week evaluation the mean body weight at necropsy of 200 ppm dosed males was significantly lower ($p<0.01$) than that of controls while mean body weights of low- and mid-dose males was similar to that of controls. At the 66-week evaluation, there was a dose-related decrease in body weights of chloraminated treated male rats. The mean body weights of high-dose male and female rats were 94 and 92% of controls, respectively. Slight decreases ($p<0.05$) in the liver and kidney weight in high-dose males and kidney-to-body weight ratios in male and female high-dose rats were related to the lower body weights in these groups.

The second NTP (1990) study was a 2-year study to determine the potential chronic toxicity or carcinogenic activity of chloraminated drinking water. B6C3F1 mice (70/sex/dose) were administered chloramine at doses of 0, 50, 100 and 200 ppm in pH 9 buffered charcoal filtered deionized drinking water. These doses were calculated based on a time-weighted average to be 0, 5.0, 8.9 and 15.9 mg/kg/day for male mice and 0, 4.9, 9.0 and 17.2 mg/kg/day for female mice. There was a dose-related decrease in the amount of water consumed by both sexes; this decrease was noted during the first week and continued throughout the study. Male and female mice had similar food consumptions as controls except the 200 ppm dose females that were slightly lower than controls. There was a dose-related decrease in mean body weights of dosed male and female mice throughout the study. Mean body weights of high-dose male mice were 10-22% lower than their control group after week 37 and the body weights of high-dose female mice were 10-35% lower after week 8. Survival of mice receiving chloraminated drinking water was not significantly different than controls. All animals were given a complete histopathologic examination at the end of the study. The authors did not attribute any clinical findings to the consumption of chloraminated drinking water.

Groups of mice (10/sex/group) were predestined for incremental sacrifice and evaluation at 15 and 66 weeks. A complete hematologic examination and necropsy were performed on all animals and a histologic examination was performed on all controls and high-dose rats at the incremental sacrifices. At 15 weeks the group mean body weights of high-dose male and female mice were 91 and 84% of controls, respectively. At the 66-week evaluation, the differences in body weight between the high-dose mice and

controls was 87% for females but 91% of controls for mid- and high-dose males. Decreases in liver weights and increases in brain- or kidney-to-body weight ratios observed in high-dose mice at 15 or 66 weeks were attributable, according to NTP, to the lower body weights in these groups.

Because specific organic N-chloramines have not yet been identified in chlorinated water or *in vivo* on ingestion of chlorinated water, Bempong and Scully (1980a) studied N-chloropiperidine (NCP), a model for the health effects of organic chloramines as a class of compounds. They found it was toxic to C57Bl/J6 mice after i.p. injection. Groups of 10 female and 10 male mice were injected with either 50, 100, 200, 300 or 400 mg/kg bw of NCP. The animals were observed daily for toxic effects, food intake and body weight. The $LD_{50}$ for NCP was slightly greater than 300 mg/kg as presented by the available data. However, the toxicity of NCP was increased when allowed to stand at room temperature in an aqueous solution, indicating that the degradation products are more toxic. The toxicity of the chloramine was less than that of the parent amine, but the toxicity of aqueous solutions of the chloramine increased on standing at room temperature for 24 hours or more.

**Developmental Toxicity**

Abdel-Rahman et al. (1982) investigated the effects of monochloramine administered in drinking water to adult female Sprague-Dawley rats. Six animals/group were administered 0, 1, 10 or 100 mg/L $NH_2Cl$ daily in the drinking water both 2.5 months before and throughout gestation. Sacrifice of rats on the 20th day of gestation was

performed for soft-tissue and skeletal examination of the progeny. Monochloramine did not produce any significant changes in rat fetuses at any dose level; in fact, there was a slight increase in fetal weight in all $NH_2Cl$ groups compared with controls.

**Reproductive Toxicity**

Eight- to 10-week-old hybrid male mice (C57Bl/J6 x DBA2) and 10-week-old inbred male golden Syrian hamsters (numbers not specified) were given daily exposures (ad libitum) to 400 mg/kg/day NCP in 0.001% ethanol for 100 days. Significant increases of abnormal sperm (either tail or head anomalies) were observed in both hamsters and mice after 5 weeks of exposure (Bempong and Scully, 1983). Another significant result among NCP-treated hamsters was reduced sperm count after 70 days of daily exposure. Greater than 80% of the hamster population was classified as sterile based on their reproductive performance. A few (number not provided) were reported as having testicular atrophy after 16 weeks of daily exposure. Gradual reductions in percent abnormal sperm were observed after the 75th day of treatment in hamsters; however, sperm counts did not increase. In mice the level of sperm abnormalities remained the same for more than 5 weeks except there was a reduction in sperm tail damage with concomitant increased anomalous sperm heads. Sperm count was also reduced in mice but did not affect percent anomalous sperm. The data support the concept that species and strain differences in sperm abnormalities are suggestive of genotoxic control of sperm development. In addition, the investigators propose that since increased levels of sperm abnormalities were observed >5 weeks after treatment, the most sensitive cells were early

spermatocytes > differentiating spermatogonia > premeiotic cells (Bempong and Scully, 1983).

Fertility studies were also undertaken wherein male mice and male hamsters were caged with two respective females after 1 week of continuous exposure as described previously (Bempong and Scully, 1983). The duration of each mating period was 7 days. Six treatment groups of 15-25 females were constituted in the following manner: 1) untreated (UT) female x UT male; 2) UT female x ethylmethane sulfonate (EMS)-treated male; 3) NCP-treated female x NCP-treated male; 4) UT female x NCP male; 5) NCP female x UT male; and 6) piperidine-treated female x piperidine-treated male. EMS served as a positive control and piperidine was included to ascertain the extent to which effects could be ascribed to the monochloramine group. Reduced fertility indices were observed on week 5 and subsequently for all matings of NCP-treated animals, except when females only were exposed (group 5). To obtain data on potential embryotoxic effects of NCP, five pregnant animals/group were allowed to reach term and the offspring were counted. Numbers of offspring/pregnancy were variable for the NCP-treated animals, but was lowest for the group (3) wherein both males and females received NCP. Treatment with NCP increased the number of uterine moles, particularly when the female was exposed. Implant frequency was reduced in all NCP treatment groups; exposure of both males and females resulted in a significantly greater reduction than treatment of either single parent (Bempong and Scully, 1983).

In a more recent study, Carlton et al. (1986) administered monochloramine by gavage at doses of 0, 2.5, 5.0 and 10 mg/kg bw/day to male (12/dose) and female (24/dose) Long-Evans rats for 66-76 days. Males were treated for 56 days and females for 14 days before mating. The administration was continued during the 10-day mating trial period, and thereafter females were gavaged with monochloramine daily throughout gestation and lactation. Males were necropsied at the end of the mating period and bled for complete blood counts and thyroid hormone levels. Their sperm was examined for normalcy and histologic examinations were performed on the reproductive tract including the testis, epididymus, prostate and seminal vesicles. Dams and some offspring were necropsied at weaning 21 days after birth of the pups. At necropsy dams were bled for complete blood counts and thyroid hormone levels. In addition, the reproductive tract was removed for histopathologic examination. Other offspring were administered chloramines after weaning until they were 28-40 days old; these pups were evaluated for vaginal patency and thyroid hormone levels. The authors reported no significant (statistical analysis not provided) differences between control and exposed rats in fertility, viability, litter size, day of eye opening, or average day of vaginal patency (day 31.8-32.6). There were no alterations in sperm count, direct progressive sperm movement, percent mobility, or sperm morphologic characteristics in adult males. Weights of male and female reproductive organs were not significantly different among test and control groups, and there were no significant morbid anatomic changes evident on tissue examination. There were no signs of toxicity, changes in hematologic parameters, or body weight suppression in adult rats of either sex at any dose level. The mean weight of the pups was unchanged from that of control litters. Based on these data, a NOAEL of 10 mg/kg/day is identified.

**Mutagenicity**

Lu Shih and Lederberg (1976) showed chloramine to be weakly mutagenic at the *trpC* locus of *Bacillus subtilis*. They further investigated the biologic and physical effects of chloramine on *B. subtilis* after treatment of the bacterial cells or the bacterial DNA. Both resulted in single-strand breaks and a few double-strand scissions at higher chloramine doses, with loss of DNA-transforming activity. Since some DNA repair-defective mutants seem to be more sensitive to chloramine, it would appear that chloramine's bacteriotoxic effect may be due in part to its ability to damage DNA.

Thomas et al. (1987) reported that monochloramine (40 $\mu$m) slightly increased the number of revertant colonies over untreated control levels in assays employing *Salmonella typhimurium* (TA97, TA100 and TA102). Positive controls were run concurrently. Lipophilic dichloramines were the most active mutagens in this study.

Fetner (1962) found that distilled water containing monochloramine produced chromosome breakage when used for soaking *Vicia faba* seeds. A 1-hour exposure to $10^{-4}$ M monochloramine produced 24% abnormal anaphases (including chromosomal bridges and fragments) in the embryonic *Vicia* roots. As there was no visible browning of the plant embryos, Fetner concluded that monochloramine produced chromosome breakage at a concentration that exhibited little evidence of tissue damage.

Meier et al. (1985) evaluated the ability of monochloramine and other oxidants to induce chromosomal aberrations (5/sex/dose) and micronuclei in the bone marrow of

CD-1 mice (4/sex/dose), and sperm-head abnormalities in male B6C3F1 mice (10/dose). Monochloramine administered by gavage at concentrations of 40, 100 and 200 mg/L showed no evidence of any significant effects in any of the tests.

Bempong and Scully (1980a) found that the organic chloramine, NCP, was weakly mutagenic in a preincubation *Salmonella* reverse mutation assay (Ames test). Mutagenic activity was detected in the absence of exogenous S9 activation in strains TA1535 and TA100. The toxicity of the chloramine appeared to overshadow its mutagenic property at doses >64 $\mu$g/plate with a concomitant reduction in the number of revertants/plate. N-Chlorodiethylamine, on the other hand, was highly toxic but nonmutagenic (Scully et al., 1983).

The effect of increasing concentrations of NCP on the toxicity, mitotic indices and chromosomal aberrations were studied in Chinese hamster cells (CHO). CHO cells exposed to NCP (0.4-2.4 $\mu$g/L) for 3 hours and analyzed during a 96-hour period proliferate less rapidly than control cells and as the concentration of NCP increased, mitotic indices were reduced and the peaks of mitotic activity shifted resulting in chromosome separation and nuclear distribution. CHO cells were exposed to increasing concentrations of NCP (0, 0.4, 0.8 and 1.2 $\mu$g/mL) for a 3-hour period, harvested 48 hours after treatment and analyzed for chromatid aberrations (breaks, exchanges, fragments, ring and centric errors). The increases in the frequency of aberrations were proportional to the increase in NCP concentration with breaks accounting for the major proportion of aberrations. Chromosomal aberrations were further investigated after a 3-hour incubation

and a 60-hour recovery period to allow for delayed mitotic activities, and increased populations of metaphases. A nonlinear dose-dependent increase in bridges, dicentrics, exchanges, fragments and ring configurations occurred (Bempong and Scully, 1980b; Bempong et al., 1981, 1986; Scully and Bempong, 1982).

Chloramine T (sodium p-toluenesulfonylchloramide) tested negative in a Salmonella reverse mutation assay (Ames test) in the presence of S-9 in strains TA98, TA100, TA1535 and TA1538 (Anderson and Styles, 1978), and negative in strains TA98, TA100, TA1535, TA1537 and TA1538 with and without S-9 fractions (Gocke et al., 1981).

Weitberg (1987) tested the ability of chloramine T (sodium p-toluene-sulfonylchloramide) to produce SCEs in cultured CHO cells. A significant dose-dependent increase was observed at $10^{-5}$ to $10^{-7}$ M and at $10^{-8}$ M when compared with controls. This effect was significantly diminished when cells were treated with methionine, a thioether, which reduces N-Cl back to the parent amine.

Süssmuth (1982) examined the mutagenicity of chlorinated solutions of several amino acids using several strains of bacteria including the recombination-deficient strain of *B.* M45 (*rec*), *Es. coli* P3478 (*polA1*), and three histidine auxotrophic strains of *S. himurium*. Chlorinated solutions of methionine, tyrosine, phenylalanine and glycine were mutagenic in more than one strain of bacteria. The active mutagens in Süssmuth's solutions were not defined clearly. Since the amino acids react rapidly in chlorinated water to form N-chloramino acids, the first group of compounds to form would be the

chloramine acids. These may be the active mutagens; however, because some of the chloramine acids are labile, their degradation by-products may be the active mutagens.

An Ames *Salmonella* assay was performed using concentrated drinking water samples collected at a pilot scale drinking water treatment plant disinfected with monochloramine (Miller et al., 1986). The reverse osmosis concentrates ranged from 0.025-1.0 mL. The mutagenic response was negative for strains TA98 or TA100 when tested with or without the metabolic activation system S9.

**Carcinogenicity**

Several initiation-promotion studies have been conducted. Although these studies are not useful for quantitative risk assessment, they may support findings in bioassay studies.

Settled, coagulated and sand-filtered Ohio River water was treated with monochloramine at 3 mg/L (Bull, 1980; Bull et al., 1982). The residual disinfectant was dissipated within 48 hours. The water was then concentrated by reverse osmosis and the concentrate subjected to a mouse skin initiation-promotion assay in SENCAR mice. A total of 1.5 mL of the reverse osmosis concentrate was applied to the backs of the mice in six doses (0.25 mL each) over a 2-week period. This was followed by thrice weekly applications of 2.5 ug of the promoting agent phorbol myristate acetate in acetone for 18 weeks. Nondisinfected water concentrates produced no tumors, while concentrated monochloramine-treated water samples induced neoplasms in 5/25 animals. Lesions

included papillomas (1/25), squamous cell carcinomas (2/25) and lung adenomas (5/25). Overall chi-square analysis comparing numbers of animals with systemic tumors indicated that the increase was not significant. Subsequent to the results published by Bull (1980), five additional experiments were undertaken using new samples of disinfected waters. Results of two of these were published by Bull et al. (1982). In initiation-promotion assays of concentrated monochloramine-treated water samples at a dose of 1.0 $\mu$g for 20 weeks, 23% and 15% of animals developed papillomas respective to the two studies. For these assays, however, papillomas were also observed in 15 and 13% of mice treated with saline and in 20% of mice exposed to nondisinfected water concentrates. The authors pointed out that the average tumor incidence of all the monochloramine concentrated water-exposed animals is almost twice that of the simultaneous controls and is more than double the historical control response. It is likely that the initiating activity of the water concentrates is not due to the monochloramine per se, but rather to organic chloramines or other materials formed as by-products of the disinfection process. The authors noted that as the reverse osmosis process used to produce the water concentrates does not concentrate low molecular weight organics, trihalomethanes were not included in these test samples.

Herren-Freund and Pereira (1986) tested chloramines for initiation-promotion activity in rat liver by using the rat liver foci bioassay. The endpoint of the assay is the occurrence of altered foci of hepatocytes. This assay uses an increased incidence of γ-glutamyltranspeptidase positive foci (GGT foci) as an indicator of carcinogenicity since initiation-promotion bioassays using GGT foci have detected both hepatic and nonhepatic

carcinogens. Male rats (authors do not clarify if Fischer 344 or Sprague-Dawley or both were used) were administered chloramine (route not specified) 14.75 mg/kg bw 24 hours following a 2/3 partial hepatectomy. Seven days after initiation, promotion by 500 ppm phenobarbital in drinking water was begun. After 10 weeks of exposure to phenobarbital, the rats were removed from the exposure to the promoter for 1 week and then sacrificed. Positive and negative controls were run concurrently. The authors reported that under conditions of this study chloramine did not initiate GGT foci.

Similar results were reported by Miller et al. (1986) when rats (10/group) were administered concentrated drinking water samples collected at a pilot scale drinking water treatment plant using monochloramine for disinfection purposes, which were concentrated (4000x or 2000x the residual concentration of 2.1 mg/L) by the macroreticular resin process. Rats were partially (2/3) hepatectomized on day 0 and treated 24 hours later with chloramines for 1 week. On day 7 the rats received 500 ppm sodium phenobarbital in their drinking water for 56 days. Chloramine-treated water did not initiate the incidence of GGT foci above that of the vehicle control group.

One of the toxicologic tests used for determining tumor-initiating potential of concentrated drinking water samples is the mouse lung adenoma assay. Concentrated drinking water samples from a pilot treatment plant using monochloramine for disinfectant purposes was administered to 6-week-old strain A mice in drinking water samples concentrated (4000x and 2000x) by the macroreticular resin process (Miller et al., 1986). The residual chloramine level before concentration was 2.1 mg/L. Both positive and

negative controls were run concurrently. The vehicle controls had 0.1 adenoma/animal and the treated animals had $\leq$0.20 adenoma/animal, indicating there was no treatment-related effect.

The National Toxicology Program (NTP, 1990) conducted a 2-year study of chloraminated drinking water in F344/N rats at doses of 0, 50, 100 and 200 ppm. This dose was calculated to be 0, 2.1, 4.8 and 8.7 mg/kg/day for males and 0, 2.8, 5.3 and 9.5 mg/kg/day for females. The purpose of this study was to determine the potential chronic toxicity or carcinogenic activity of chloraminated water. The water from all treatment groups was charcoal filtered and deionized to remove organic substances and other residues.

According to the authors there were no neoplasms or nonneoplastic lesions in rats of the 2-year study that were attributable to the consumption of chloraminated drinking water. The incidence of mononuclear cell leukemia was slightly increased relative to that of controls in the mid- and high-dose range. Although female rats receiving chloraminated water had significantly greater leukemia incidence in the high-dose group than the controls, there was no clear dose response and the incidence of leukemia in the female control group (16%) was less than the untreated historical controls (25%). There was also no evidence of a reduced latency in the occurrence of leukemia in female rats consuming chloraminated water. However, because of the marginal statistical significance the increased incidence of leukemia was considered equivocal evidence of

carcinogenic activity in female rats. There was no evidence of carcinogenic activity of chloraminated drinking water for male rats.

The National Toxicology Program (NTP, 1990) conducted another 2-year study of chloraminated drinking water in B6C3F1 mice at doses of 0, 50, 100 and 200 ppm. These doses were calculated to be 0, 5.0, 8.9 and 15.9 mg/kg/day for males and 0, 4.9, 9.0 and 17.2 mg/kg/day for females. The purpose of this study, as with the rat study, was to determine the potential chronic toxicity or carcinogenic activity of chloraminated water. The water from all treatment groups was charcoal filtered and deionized to remove organic substances and other residues.

Renal tubular cell adenomas occurred in two high-dose and one low-dose male mice; focal tubule hyperplasia was seen in one low-dose, three mid-dose and one high-dose male. Hyperplasia was also observed in male control animals. There were no other lesions in the kidneys of male or female mice receiving chloraminated water. Histopathology evaluation of the kidneys failed to identify any difference in the incidence or severity of renal tubular atrophy, dilation, regeneration, focal mineralization, or protein casts. Step sections of the kidneys of male mice did not provide supportive evidence of a chemical effect. There were no neoplasms or nonneoplastic lesions in mice in the 2-year study that were attributable to the consumption of chloraminated drinking water, according to the authors. There was no evidence of carcinogenic activity of chloraminated drinking water for male or female mice.

**Summary and Discussion**

Several short-term studies showed no observed adverse hematologic effects in mice, rats and monkeys (Moore et al., 1980; Bercz et al., 1982; Bull, 1980). In A/J mice administered chloramine solutions between 2.5 and 200 mg/L (pH 8.9) for 30 days, the only observable effect was a slight increase in hematocrit (Moore et al., 1980). In another study of similar duration (45 days) rats treated with 10, 50 or 100 mg/L monochloramines experienced a decrease in the amount of methemoglobin present in the blood, the opposite of what was expected (Bull, 1980). Monochloramine in drinking water for 6 weeks at 100 mg/L resulted in no detectable effects on 18 hematologic tests of 12 African Green monkeys (Bercz et al., 1982). The results from the three studies described above are somewhat conflicting in that similar dose levels and duration produced different changes in hematologic parameters (i.e., increased hematocrit, decreased methemoglobin in blood) while others produced no detectable effects. In one longer-term study in which blood glutathione levels were decreased after 12 months of exposure to monochloramine, there was a lack of dose- and time-dependency in the observed effects (Abdel-Rahman et al., 1984).

One possible reason for the varying observations between laboratories may be the conditions under which the solutions of monochloramine are generated. It is understood that, as the pH of a solution of monochloramine is lowered, some of the monochloramine is converted to dichloramine. To avoid this, solutions are frequently buffered to pH 8 to avoid dichloramine formation. However, in doing this, buffer concentrations may be exerting an unusually strong effect on the reactions of disinfectants in the stomach where

enzyme activity is usually greatest (lower pH). The pH and buffer concentrations of the monochloramine solutions used in toxicity studies may vary widely from laboratory to laboratory.

A draft report by GSRI (1981) investigated the effects in rats and mice administered drinking water containing 0, 25, 50, 100, 200 or 400 mg/L monochloramine for 91 days. Using water and food consumption data provided in the report, corresponding respective doses were 2.5, 4.9, 10.2, 18.8 and 40.7 mg/kg/day for male rats; 3.8, 6.5, 13.8, 26.6 and 53.9 mg/kg/day for female rats; 4.9, 8.3, 14.5, 31.3 and 50.7 mg/kg/day for male mice; and 7.7, 12.1, 21.9, 34.6 and 88.5 mg/kg/day for female mice. Animals experienced decreased body weight gain and liver damage at 200 and 400 mg/L monochloramine in rats and 100, 200 and 400 mg/L monochloramine in mice. Based on these observations, the investigators suggested a NOEL of 50 mg/L or ~8.3 mg/kg/day monochloramine.

In addition monochloramine at 9, 19 and 38 ppm in drinking water may exert immunotoxic effects to male Sprague-Dawley rats. Significant ($p<0.05$) reduction of spleen weight (38 ppm), decreased antibody synthesis (9 and 19 ppm) and augmented PGE2 production (19 and 38 ppm) were reported (Exon et al., 1987). The biologic importance of effects on the immune system as a factor in the toxicity of monochloramines is not clear.

Daniel et al. (1990) investigated the effects of monochloramine administered for 90 days in the drinking water of Sprague-Dawley rats at 0, 25, 50, 100 and 200 mg/L. Based on food and water consumption data, doses were calculated to be 0, 1.8, 3.4, 5.8 and 9.0 mg/kg/day for males and 0, 2.6, 4.3, 7.7 and 12.1 mg/kg/day for females. For males and females the average weight gain at 200 mg/L dose was 51% of controls. Reductions in absolute and relative organ weights occurred at 200 mg/L. The authors concluded that 100 mg/L is considered the NOAEL.

Daniel et al. (1991) also investigated the effects of monochloramine administered for 90 days in the drinking water of B6C3F1 mice at 0, 12.5, 25, 50, 100 and 200 mg/L. Based on food and water consumption data, doses were calculated to be 0, 2.5, 5.0, 8.6, 11.1 and 15.6 mg/kg/day for males and 0, 2.8, 5.3, 9.2, 12.9 and 15.8 mg/kg/day for females. Based on decreases in liver, heart and lung weights in males and liver, heart and spleen weights in females at 100 mg/L and decreased weight gain and food consumption in both sexes and reduced water consumption in females, the 50 mg/L concentration is considered the NOAEL.

NTP (1990) conducted two studies for 2 years (F344/N rats and B6C3F1 mice) to determine the potential chronic toxicity for chloraminated drinking water. Chloramine was administered at doses of 0, 50, 100 and 200 ppm. These doses were calculated to be 0, 2.1, 4.8 and 8.7 mg/kg/day for male rats and 0, 2.8, 5.3 and 9.5 mg/kg/day for female rats, and 0, 5.0, 8.9 and 15.9 mg/kg/day for male mice and 0, 4.9, 9.0 and 17.2 mg/kg/day for female mice. Mean body weights of 200 ppm dosed rats were lower than controls. Slight

decreases in organ-to-body weight ratios in high-dose rats were related to the lower body weights. There was a dose-related decrease in mean body weights of dosed male and female mice throughout the study. No clinical changes were attributable to chloraminated drinking water in the rat or mouse study.

Studies suggest that flour treated with trichloramine may cause "canine hysteria" but one study suggests that this is a species-specific phenomenon in dogs (Mellanby, 1946; Silver et al., 1947a,b,c; Newell et al., 1947) and does not affect humans (Pollock, 1949).

Few studies have been conducted on organic N-chloramines. Studies conducted with N-chloropiperidine suggest that organic chloramines may be toxic, but determination of their environmental significance will rely on identification of specific organic N-chloramines actually formed on chlorination of natural waters (Bempong and Scully, 1980a).

Monochloramine was not teratogenic in mature female Sprague-Dawley rats exposed to 1, 10 or 100 mg/L in drinking water nor did 40, 100 and 200 mg/L solutions induce sperm-head anomalies in B6C3F1 mice (Abdel-Rahman et al., 1982).

In a more recent study there were no significant differences in fertility, viability, litter size, day of eye opening or day of vaginal potency between control and exposed Long-Evans rats given $\geq$10 mg/kg chloramines. There were no alterations in sperm

count, direct progressive sperm movement, percent mobility or sperm morphologic characteristics in adult males. Weights of male and female reproductive organs were not significantly different among test and control groups.

Data on the mutagenicity of chloramines are inconclusive. A number of studies suggest that monochloramine and some organic N-chloramines and N-chloramino acids may be weakly mutagenic. Monochloramine has been found to be marginally mutagenic in *Bacillus subtilis* bacteria (Lu Shih and Lederberg, 1976) and in *Vicia faba* plant seeds (Fetner, 1962). It was responsible for cellular hypertrophy, increased mitotic figures and bizarre chromatin patterns in B6C3F1 mice exposed to 200 and 400 mg/L in drinking water (Wolfe et al., 1984). Thomas et al. (1987) reported monochloramine (40 um) slightly increased the number of revertant colonies over untreated control levels in assays employing *Salmonella typhimurium* (TA97, TA100 and TA102). In addition, monochloramine showed no evidence of producing spermhead abnormalities in B6C3F1 mice or inducing chromosomal aberrations and micronuclei in bone marrow of CD-1 mice (Meier et al., 1985).

The organic chloramine, N-chloropiperidine, was found to be marginally mutagenic in the reverse mutation plate incorporation assay (Ames test). It was cytotoxic and cytostatic in CHO cells and produced chromosomal aberrations, the frequency of which was proportional to the concentration of the compound (Bempong and Scully, 1980b; Bempong et al., 1981, 1986; Scully and Bempong, 1982). It produced SCEs in CHO cells, but not in baby hamster kidney cells (Scully et al., 1983). The analogous

chloramine, N-chlorodiethylamine, was more toxic but nonmutagenic (Scully et al., 1983). When the synthetic N-Cl compound chloramine T (sodium p-toluenesulfonylchloramide) was tested, SCEs were significantly increased in a dose-dependent manner in CHO cells (Weitberg, 1987).

Data indicate that chloramine per se is not an initiator (Bull et al., 1982; Bull, 1980; Herren-Freund and Pereira, 1986). Two-year studies by the National Toxicology Program (NTP, 1990) have shown equivocal evidence of carcinogenic activity in female rats that is due to the slightly increased incidence of mononuclear cell leukemia compared with that of concurrent controls, although incidence in historical controls was higher. There was no evidence of carcinogenic activity that isdue to chloraminated drinking water in male rats or in male or female mice. Until the carcinogenicity of chloramines is further delineated, it is categorized in group D, not classifiable as to human carcinogenicity, meaning that there is inadequate human and animal evidence of carcinogenicity (U.S. EPA, 1986).